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(54) Title: AMINO-SALICYLIC ACID DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS

(57) Abstract

(5-Acylamino-2-hydroxy)benzoic acid and salts thereof with imidazole, substituted imidazole, lysine or methyl-glucamine are endowed with remarkable antiinflammatory, antiaggregating and antithrombotic properties.

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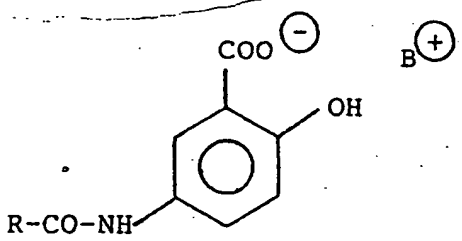
Amino-Salicylic acid derivatives and pharmaceutical compositions

The present invention refers to (5-amino-2-hydroxy)benzoic acid derivatives having formula I



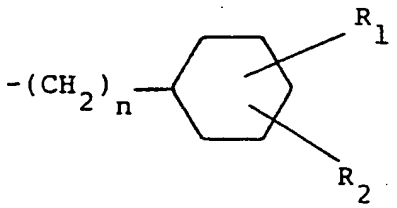
(I)

wherein:

$B^{\oplus}$ is the imidazolium or C- or N-substituted imidazolium cation, lysine or similar basic aminoacids or methylglucamine;

R represents:

- hydrogen or a linear C_1-C_{25} alkyl chain, optionally substituted by one or more chlorine or fluorine atoms, free, etherified or esterified hydroxy groups, carboxy, carboxyalkyl, aminocarbonyl or N-substituted aminocarbonyl groups, one or more of the $-CH_2-$ groups being optionally substituted by keto groups;
- a chain of formula:



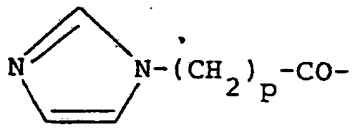
wherein n is an integer from 1 to 10 and R_1 and R_2, which may be the same or different, are H, halogens, $-OR_3$ or $COOR_3$ groups wherein R_3 is hydrogen or C_1-C_5 lower alkyl;

- a chain of formula:

$$-(CH_2)_m-Het$$

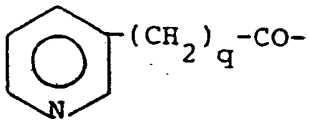
wherein m is an integer from 0 to 20 and Het is an optionally substituted 5- or 6-membered heterocyclic group containing one or more N, O or S atoms such as pyrrole, pyridine, furan, pyran, thiophene, oxazole, isoxazole, imidazole, pyrazole, thiazole groups;

- a chain of formula:



wherein p is an integer from 0 to 16;

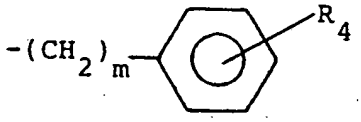
- a chain of formula:



wherein q is an integer from 1 to 16;

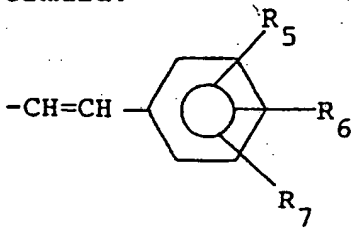
- an aryl or aralkyl residue such as phenyl; phenyl substituted by one or more fluorine or chlorine atoms, fluoroalkyl, alkoxy, alkoxycarbonyl, C_1-C_4 lower alkyl, amino, dialkylamino, hydroxy, cyano groups or by groups of formula $NHCOR_3$ wherein R_3 has the above defined meanings; diphenyl; naphtyl groups;

- a chain of formula:



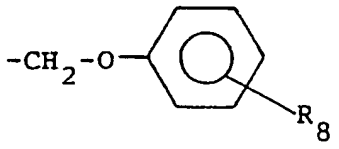
wherein m has the above defined meanings and R_4 is hydrogen or a linear or branched, saturated or unsaturated, C_1-C_{20} alkyl group;

- a chain of the formula:



wherein R_5, R_6 and R_7, which may be the same or different, are H, OR_3 (R_3 having the above defined meanings), NH_2, $NHCOR_3$, chlorine or fluorine atoms, fluoroalkyl groups;

- a chain of formula:



wherein R_8 is hydrogen, lower alkyl, fluorine or fluoroalkyl;

- a linear or branched chain of the formula:

$$-(CH_2)_n-X-\underset{\displaystyle R_3}{\underset{|}{CH}}-(CH_2)_{n'}-Y-$$

wherein R_3 and n have the above defined meanings, n' is an integer from 1 to 10 and X and Y are an oxygen, nitrogen, sulphur atom or a CH_2 group;

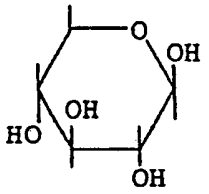
- a chain of formula:

$$-(CH_2)_r-S-R_3$$

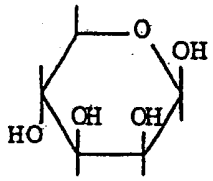
wherein r is an integer from 1 to 3 and R_3 has the above

defined meanings;

- an aminoacid residue, namely L-leucyl, α or γ-L-glutamyl- in a free form or protected with the conventional amine protecting group, such as BOC;
- an Arg-Pro-D(Phe) chain or the like;
- an uronic residues of formula:



or



Another object of the invention is provided by a process for the preparation of the compounds I as well as by pharmaceutical compositions containing them as the active principle.

The acids 5-(2,4-dichlorobenzoylamino)-2-hydroxy benzoic, 5-(cyclohexylmethylamino)-2-hydroxy benzoic, 5-(linoleylamino)-2-hydroxy benzoic, 5-(arachidylamino)-2-hydroxy benzoic, 5-(arachidonylamino)-2-hydroxy benzoic, 5-(2,6 or 3,5-difluoro-phenyl)-2-hydroxy benzoic, 5-(4-cyclohexyl-butanoylamino)-2-hydroxy benzoic, 5-[2-(3-pyridyl)acetylamino]-2-hydroxy benzoic, 5-[4-(phenyl)benzoylamino]-2-hydroxy benzoic, 5-(m-trifluoromethyl-cinnamoyl)-amino-2-hydroxy benzoic, 5-[8-(1-imidazolyl)-octanoyl]amino-2-hydroxy benzoic are per se new and are therefore comprised within the scope of the present invention, as well as the salts thereof with pharmacologically acceptable organic or inorganic bases and the pharmaceutical compositions containing them.

On the other hand, while the imidazole salts I are of course new, some of the corresponding acids (the anio-

nic component) are known, for instance from EP-A-45955, Ger. Offen. No. 2,031,227, 2,919,545, 2,920,292, Japan Kokai No. 78-9651, Biochem. Biophys. Res. Commun. V. 101, 258, 1981 and Biomed. Mass Spectrom. 11, 539, 1984: no pharmacological activity thereof has been however described.

It has now been found that also these known compounds are endowed with surprising and advantageous pharmacological properties.

A further object of the invention is therefore provided by pharmaceutical compositions containing as the active principle said known acids, which will be hereinafter specifically defined.

The preparation of the compounds of the invention is carried out starting from 5-amino-salicylic acid, which, in the presence of a suitable base (pyridine, triethylamine etc.), optionally diluted in a suitable solvent, is treated with equimolar amounts of an activate derivative, such as the acyl chloride or anhydride, of an acid of formula RCOOH wherein R has the above defined meanings. After stirring at the room temperature or under heating, a mixture of N,O-diacyl and of N-acyl product is usually obtained which is subjected to selective hydrolysis of the O-acyl group in extremely mild conditions so as to respect both the N-acyl group and the nature of the acyl group itself. Said method is characterized by treating the acyl derivatives mixture, recovered from the reaction medium and dissolved in suitable solvent, with catalytic amounts of imidazole base in the presence of minor amounts of water. After stirring at room temperature, for different

times according to the considered acyl group, the recovery of the N-acyl derivative is carried out by solvent evaporation and subsequent recrystallization from a suitable solvent.

The imidazole or substituted imidazole salts, as well as the pharmacologically acceptable metals or other organic bases salts, are prepared by mixing in a suitable solvent equimolar amounts of the corresponding acid and base. The recovery of the salt is carried out either by spontaneous precipitation from the reaction medium or by solvent evaporation under vacuum or by addition to the medium itself of a miscible precipitating solvent.

The following examples further illustrate the invention, without limiting the scope thereof.

EXAMPLE 1

1) 5-(2,4-Dichlorobenzoylamino)-2-hydroxy-benzoic acid

20.95 Grams (0.1 mole) of 2,4-dichlorobenzoyl-chloride are slowly added to a solution of 15.31 g (0.1 mole) of 5-amino-salicylic acid in 150 ml of anhydrous pyridine under stirring, in the dark and in nitrogen atmosphere. The solution is then poured in water-ice, filtered under reduce pressure and the obtained precipitate is washed with water to neutrality and dissolved in humid methanol. 0.5 Grams of imidazole base are then added, and the mixture is stirred at the room temperature for 3 hours. The solvent is distilled off under vacuum and the residue is taken up with ethyl acetate, washed with acidic H_2O (HCl) then with water to neutrality and dried on sodium sulphate. After solvent's evaporation, the residue is crystallized from methanol, yielding 17.49 g (53,63%), m.p. 233-235°C; I.R.:

elemental analysis, found (calc.): C = 51.74 (51.55); H = 2.85 (2.78); N = 4.21 (4.29).

1a) Imidazole salt

10 Grams (30.66 mmoles) of the product 1 are dissolved in 100 ml of methanol and added with 2.09 g (30.66 mmoles) of imidazole. The mixture is stirred for 3 hours and the solvent is then removed under reduced pressure. The residue is crystallized from methanol-water. 10.65 Grams of 1a are obtained (88.11%) having a melting point of 87-89°C; I.R. : 3140, 1650, 1585, 1320 cm^{-1}; U.V.: 233, 253, 325 nm; elemental analysis, found (calc.): C = 50.83 (51.80); H = 3.47 (3.23); N = 10.72 (10.66).

EXAMPLE 2

2) 5-Hexadecanoylamino-2-hydroxy benzoic acid

15.31 Grams (0.1 mole) of 5-amino-salicylic acid are dissolved in 300 ml of anhydrous pyridine. After cooling to 0°C, under nitrogen and in the dark, 41.23 g (0.15 moles) of hexadecanoyl chloride are slowly added under stirring. When the addition is over, stirring is continued for 3 hours, the mixture is poured in 100 ml of water-ice and then extracted with ethyl acetate. The organic phase is washed with diluted hydrochloric acid, water and the solvent is evaporated under reduced pressure. The residue is taken up with 100 ml of acetone and 10 ml of water, 0.68 g of imidazole base are added and the mixture is stirred overnight. After solvent evaporation under reduced pressure, the residue is treated with ethyl acetate. The organic phase is washed with water, diluted hydrochloric acid and water to neutrality. After drying on sodium sulphate and filtration, the solvent is evaporated under

vacuum. The residue is crystallized from ethanol/water. Yield: 28 g (72%).

The product melts at 185-187°C; I.R. : 3500, 3290, 1680, 1650, 1540, 1310 cm^{-1}; U.V.: 223, 250, 325 nm; elemental analysis, found (calc.): C = 70.13 (70.55); H = 9.41 (9.52); N = 3.42 (3.58).

2a) Imidazole salt

11.74 Grams (30 mmoles) of the compound obtained in 2 are dissolved in 100 ml of acetone and added with 2.04 g (30 mmoles) of imidazole base. After stirring at room temperature for 5 hours, the solvent is evaporated under reduced pressure and the residue is crystallized from methanol-water.

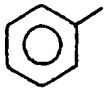
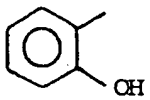
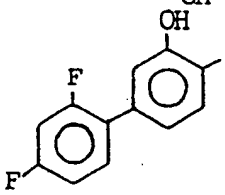
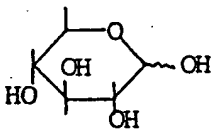
11.35 Grams (82.34%) are obtained. M.p. 132-134°C; I.R.: 3310, 1650, 1525, 1300 cm^{-1}; U.V.: 233, 253, 325 nm; elemental analysis, found (calc.): C = 67.58 (67.95); H = 8.78 (8.99); N = 8.82 (9.14).

EXAMPLES 3-23

Using the same methods above described, starting from the suitable acyl derivatives, the compounds reported in the following table were prepared.

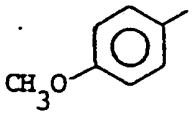
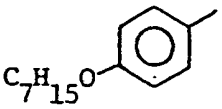
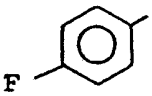
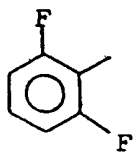
The integers followed by an a) designate the imidazole salts while the free acids are designated by progressive integers. The melting points are in °C and the I.R. values are in cm^{-1}. All the compounds have elemental analysis in agreement with the calculated values.

TABLE 1

R	Ex.No.	Melting point; I.R.
$CH_3-CH(CH_3)-CH_2$	3	p.f.: 194-196.
	3a	p.f.: 143-145; I.R.: 3300, 1660, 1530, 1305.
	4	p.f.: 212-214.
	4a	p.f.: 155-156; I.R.: 3280, 1645, 1540, 1300.
$HOOC-CH_2-CH_2-$	5	p.f.: 204(**).
	5a	p.f.: 150; I.R. 3325, 1700, 1640, 1550, 1300.
	6	p.f.: 257-259(***).
	6a	p.f.: 145-150; I.R.: 3270, 1640, 1530, 1310.
	7	p.f.: 233-235.
	7a	p.f.: 178-181; I.R.: 3030, 1650, 1520, 1305.
	8	p.f.: 256-257.
	8a	p.f.: 163-165. I.R.: 3025, 1660, 1500, 1300.
$EtOOC-NH-CH_2-$	9	p.f.: 153-155.
	9a	p.f.: 102-103; I.R.: 3100, 1650, 1510, 1330.
$EtOOC-NH-(CH_2)_5-$	10	p.f.: 161-163.
	10a	p.f.: 110-112; I.R.: 3310, 1640, 1525, 1300.
$HOOC-CH_2-CH_2-CH(NH-COOEt)-$	11	p.f.: 218-220.
	11a	p.f.: 167-170; I.R.: 3300, 1650, 1520, 1295.
	12	p.f.: 181-182.
	12a	p.f.: 155-156; I.R.: 3320, 1640, 1510, 1305.

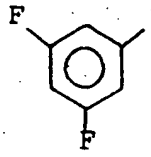
- continued -

TABLE 1 (follows)

R	Ex.No.	Melting point; I.R.
H-	13	p.f.: 236-238.
	13a	p.f.: 150-152; I.R.: 3310, 1630, 1530, 1300.
n-C17H35- (stearyl)	14	p.f.: 198-199 (*).
	14a	p.f.: 143-145; I.R.: 3300, 1650, 1560, 1310.
	15	p.f.: 235-240.
	15a	p.f.: 181-185; I.R.: 3310, 1650, 1580, 1315.
	16	p.f.: 185-186.
	16a	p.f.: 135-135; I.R.: 3310, 1640, 1525, 1300.
	17	p.f.: 230-232(****).
	17a	p.f.: 170; I.R.: 3100, 1640, 1570, 1305.
CH_3-	18	p.f.: 213-214(***).
	18a	p.f.: 151-152; I.R.: 3300, 1640, 1535, 1305.
linoleyl	19	p.f.: 159.
	19a	p.f.: 118-120; I.R.: 3320, 1650, 1530, 1300.
n-C19H39- (arachidyl)	20	p.f.: 160.
	20a	p.f.: 112-114; I.R.: 3320, 1650, 1525, 1300.
arachidonyl-	21	p.f.: 154.
	21a	p.f.: 127; I.R.: 3320, 1650, 1530, 1300.
	22	p.f.: 218-220.
	22a	p.f.: 115-119; I.R.: 3140, 1650, 1580, 1320.

- continued -

TABLE 1 (follows)

R	Ex.No.	Melting point; I.R.
	23	p.f.: 215-219.
	23a	p;f.: 97-98; I.R.: 3140, 1650, 1580, 1320.

(*) Known from E.P. 45,955;

(**) " " Biochem. Biophys. Res. Commun.-v.101, 258, 1981;

(***) " " Ger. Offen. 2,031,227;

(****)" " Biomed. Mass Spectrom, v. 11, 539, 1984.

EXAMPLES 24-28

According to the same methods of the previous claims, the following 5-acyloyl-amino-salicylic acids were prepared (I.R. values in cm^{-1} and elemental analysis in agreement):

24- 2-hydroxy-5-(4-cyclohexyl-butanoyl)-amino benzoic acid; m.p. : 196-198°C; I.R.: 3500, 3250, 1680, 1650, 1540, 1310;

25- 2-hydroxy-5-(2-(3-pyridyl)-acetyl)-amino benzoic acid; m.p. : 221-223°C; I.R.: 3510, 3260, 1680, 1640, 1540, 1300;

26- 2-hydroxy-5-(4-phenyl-benzoyl)-amino benzoic acid; m.p. : 178-180°C; I.R.: 3520, 3260, 1680, 1640, 1530, 1300;

27- 2-hydroxy-5-(m-trifluoromethyl-cinnamoyl)-amino benzoic acid; m.p. : 162-168°C; I.R.: 3500, 3250, 1660, 1635, 1500, 1300;

28- 2-hydroxy-5-(8-(1-imidazolyl)-octanoyl)-amino ben-

zoic acid; m.p. : 183-186°C; I.R.: 3510, 3260, 1680, 1635, 1510, 1300.

The corresponding imidazolium salts as well as other pharmaceutically acceptable salts of the above compounds are prepared according to the above described methods.

BIOLOGICAL ACTIVITIES

The hereinabove mentioned compounds have been tested on in vitro and in vivo assays, with the aim of giving evidence to their potential biological activities.

Soy lipoxygenase inhibition activity

This assay allows to show the presence of an inhibitory activity on soy lipoxygenase considered as a model of the human enzyme. In mammals, this enzymatic system promotes the arachidonic acid transformation in leukotrienes A4 and B4. These compounds are indicated to be fundamentally responsible for the flogosis. Namely, the leukotrienes A4 and B4, show a relevant pro-inflammatory activity in the bowell inflammatory disease and in the Crohn' disease.

The soy lipoxygenase (E.C. 1.13.11.12), has been tested according to the method of Axelrod et al. (Axelrod B. - Cheesbrough T.H., Laakso S. in Methods in Enzymology, vol. 71, pag. 441, 1981 - Academic Press N.Y.), in the presence and in the absence of the products to be assayed, using nordihydroguaiaretic acid as test reference, at room temperature.

In Table 2, as not limiting example, the results obtained with the compounds 1, 1a to 18, 18a are shown.

In this and in the following tables, as in Table 2,

the integers designate the free acids while the integers followed by the letter a relate to the corresponding imidazolium salts.

TABLE 2

Compound	Inhibition (%)	Concentration ($Mx10^{-6}$)
1	30	150
1a	40	150
2	63	75
2a	95	75
3	22	300
3a	35	300
4	16	150
4a	28	150
5	18	150
5a	31	150
6	20	150
6a	37	150
8	25	150
8a	37	150
9	10	150
9a	25	150
10	12	150
10a	18	150
11	12	150
11a	21	150
12	11	150
12a	18	150

- continued -

TABLE 2 (follows)

Compound	Inhibition (%)	Concentration ($Mx10^{-6}$)
13	14	150
13a	22	150
14	42	75
14a	78	75
15	24	150
15a	38	150
16	35	150
16a	51	150
17	26	150
17a	42	150
18	50	250
18a	82	250

Activity on platelet aggregation and thromboxane A2 production

Measurements were carried out on in vitro tests with citrated platelet-rich plasma (P.R.P.), obtained from New-Zealand rabbits. Platelet aggregation was carried out according to the method of Born (Born G.V.R. - Nature, vol. 162, 67) using arachidonic acid 0.25 mM as aggregating agent.

The inhibition activity was evaluated as ED50 (in mM), i.e. the dose which antagonizes 50% of the aggregating effect of arachidonic acid.

The thromboxane A2 production was measured by a bioassay test according to Moncada et al. (Moncada S.,

Ferreira S.H., Vane J.R. - Adv. Prost. & Thromboxanes Res. - Frolich J.C. Ed., Vol. 5, 211, 1978 - Raven Press). At scheduled times after the addition of arachidonic acid, 200 µl of P.R.P. was bioassayed for the TXA2 production and prostaglandin-like activity, on a tissue sequence (cascade), composed of a spiral strip of rabbit aorta and a stomach fundus strip of rat.

The inhibition activity of the tested compounds on TXA2 production was evaluated as ED50 (in M), i.e. the concentration able to decrease the contracturant effects of TXA2 on tissues.

The tested compounds were dissolved in Tween 80 and added to the P.R.P. at increasing concentrations, until the determination of the ED50 was achieved.

In Table 3, as not limiting example, the results obtained using some of the compounds of the invention are reported.

TABLE 3

Inhibition (ED50, in M) on arachidonic acid induced:

Compound	Platelet aggregation	TXA2 production
2	5×10^{-3}	5×10^{-3}
2a	6×10^{-5}	5×10^{-6}
4	$> 10^{-3}$	$> 10^{-3}$
4a	4×10^{-5}	5×10^{-5}
5	$> 10^{-3}$	$> 10^{-3}$
5a	10^{-3}	6×10^{-4}
6	$> 10^{-3}$	$> 10^{-3}$
6a	5×10^{-4}	4×10^{-4}
13	$> 10^{-3}$	$> 10^{-3}$
13a	1×10^{-4}	1×10^{-4}
18	$> 10^{-3}$	$> 10^{-3}$
18a	3×10^{-4}	1×10^{-4}

ANTIINFLAMMATORY ACTIVITY ON NON-IMMUNE AND IMMUNE INFLAMMATION

1 - Carrageenin induced pleurisy in rats (non-immune inflammation)

The test has been performed according to Di Rosa et al. (Di Rosa M., Giround J.P., Willoughby D.A. - J. Path. Bact., vol. 104, 15, 1971).

A 1% solution (0.15 ml) of carrageenin in 0.9% NaCl, was injected into the pleural cavity of Sprague-Dawley rats, weighing about 250 g. Six hours later, the animals were sacrificed, the pleural exudate volumes were measured and the leukocytes total number was counted by a micro-cell-counter, being the cavity rinsed by 0.5 ml of a sali-

ne medium.

The % inhibition of leukocytes total number was calculated versus control animals. The assayed compounds were administered orally, 1 mM/kg, 30' before the carrageenin injection in the pleural cavity. In Table IV, as not limiting example, the results obtained with some of the compounds of the invention are reported.

TABLE 4

% Inhibition on:

Compound	Exudate volume	Leukocytes number
1	-25	-12
1a	-46	-45
2	0	0
2a	-10	-10
3	-15	-10
3a	-40	-42
4	-8	-18
4a	-13	-48
5	-5	-10
5a	-12	-22
6	-10	-5
6a	-30	-30
7	-15	-25
7a	-35	-45
8	-15	-15
8a	-21	-22
9	-5	-5
9a	-12	-15

- continued -

TABLE 4 (follows)

Compound	Exudate volume	Leukocytes number
10	-5	-5
10a	-10	-10
11	-15	-14
11a	-21	-31
12	-10	-5
12a	-18	-16
13	-10	-16
13a	-28	-29
14	-5	-10
14a	-10	-22
15	-12	-20
15a	-32	-35
16	-15	-21
16a	-30	-38
17	-16	-20
17a	-31	-36
18	-10	-0
18a	-47	-40
23	-28	-15
23a	-50	-51
24	-16	-15
24a	-35	-44
25	-10	-32
25a	-27	-44
26	-15	-21
26a	-32	-38

- continued -

TABLE 4 (follows)

Compound	Exudate volume	Leukocytes number
27	-18	-10
27a	-31	-28
28	-16	-25
28a	-33	-40
29(*)	-16	-16
29a	-22	-30
30(**)	-15	-16
30a	-25	-31
31(***)	-10	-16
31a	-22	-25

(*) L-Leucyl-5-amino-salicylic, described in Ger. Offen. 2,919,545;

(**) γ-L-Glutamyl-5-amino-salicylic, described in Ger. Offen. 2,920,292;

(***) Aceto acetyl-5-amino-salicylic, described in Japan Kokai 78-9651.

2 - Reserve passive Arthus reaction in rat paws (Immune inflammation)

The assay has been performed according to Gemmel et al. (Gemmel D.K. Cottney J., Lewis A.J. - Agents Actions, vol. 9, 107, 1979). 1 Ml of a rabbit anti-bovine-albumin serum (freeze-dried antibodies, dissolved in 2 ml of 0.9% NaCl) was injected into the caudal vena of Sprague-Dawley male rats.

30' Later, 0.025 mg of bovine albumin (in 0.1 ml saline) was injected in the subplantar paw. The volume of the paw was measured 5 hours later, by a mercury plethysmometer.

The tested compounds were orally administered 3 hours before the bovine albumin treatment. The % inhibition of the rat foot volume increase was calculated in confront to the increase of the foot volume of untreated animals. The results obtained with some of the compounds of the invention are reported in Table 5.

TABLE 5

Compounds	Adm. route	% oedema inhibition at 5 hrs.
Na-5-ASA(*)(153)	oral	+10
Na-5-ASA (100)	i.v.	-6
1 (326)	oral	-20
1 (100)	i.v.	-24
1a (394)	oral	-31
1a (100)	i.v.	-36
2 (391)	oral	-9
2a (460)	oral	-11
3 (237)	oral	-15
3 (100)	i.v.	-26
3a (305)	oral	-22
3a (100)	i.v.	-31
4 (277)	oral	-18
4 (100)	i.v.	-34
4a (345)	oral	-19
4a (100)	i.v.	-38
5 (253)	oral	-10
5 (100)	i.v.	-22
5a (321)	oral	-27
5a (100)	i.v.	-33
6 (257)	oral	-25
6 (100)	i.v.	-27
6a (325)	oral	-31
6a (100)	i.v.	-31
9 (282)	oral	-15
9a (350)	oral	-21

- continued -

TABLE 5 (follows)

Compounds	Adm. route	% oedema inhibition at 5 hrs.
10 (338)	oral	-21
10a (406)	oral	-23
11 (354)	oral	-10
11a (422)	oral	-18
12 (329)	oral	-10
12a (397)	oral	-10
13 (181)	oral	-5
13 (100)	i.v.	-8
13a (249)	oral	-18
13a (100)	i.v.	-27
14 (419)	oral	-3
14a (487)	oral	-9
15 (287)	oral	-25
15a (355)	oral	-32
16 (371)	oral	-24
16 (100)	i.v.	-30
16a (439)	oral	-32
16a (100)	i.v.	-36
17 (275)	oral	-28
17a (343)	oral	-38
18 (195)	oral	-3
18 (50)	i.v.	-5
18a (263)	oral	-20
18a (100)	i.v.	-35

(*) Na-5-ASA: 5-amino salicylic acid sodium salt
The numbers in parenthesis indicate the administered dose in mg/kg.
The orally administrations are equivalent to one mM/kg for each tested compound.

3 - Acetic acid bowel inflammation in rats (non immune bowel inflammation)

The assay has been performed according to Sharon (Sharon P., Stenson W.F. - Gastroenterology, vol. 88, 55, 1985).

Considering the nature of the assay, the test was performed mainly on the compounds of the invention not well absorbed, according to the results, obtained in the previous tests. It should be noted, however, that all the claimed derivatives can be usefully applied in the therapy of the bowel inflammation and in the Crohn' disease.

The results are reported in the following Table 6.

The administered doses (via intra-bowel, during the bowel ligature and the local injection of the acetic acid) were 0.5 mM/kg, for all the tested compounds, dispersed in carboxymethylcellulose. The % reduction of the ulceration index has been calculated versus untreated animals.

TABLE 6

Compounds	% Reduction of the ulceration index
2	-36
2a	-51
14	-38
14a	-58
19	-42
19a	-65
20	-42
20a	-65

Acute toxicity

The compounds of Table 1 have been subjected to the acute toxicity test in mice, by the oral route, in carboxymethylcellulose suspensions. All the LD_{50} proved to be higher than 1600 mg/kg.

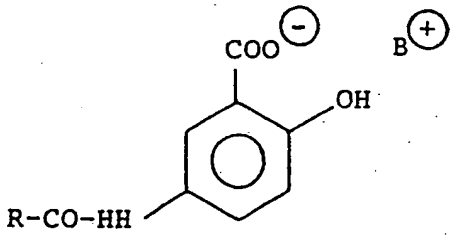
The present invention refers also to all the industrial applicable aspects connected with the use of the compounds I and of the corresponding free acids as therapeutic agents. An essential aspect of the invention is therefore provided by pharmaceutical compositions containing, as the active principle, predetermined and therapeutically effective amounts of at least one of the above compounds in addition to conventional excipients and/or carriers.

The compositions of the invention can be administered by the oral, parenteral, rectal or topical route, for instance in form of tablets, capsules, syrups, sachets, solutions, vials, bottles, suppositories.

The doses will be dependent on the patient's weight, age and conditions and will be anyhow ranging from 50 to 1000 mg, from 1 to 4 times a day.

CLAIMS

1.



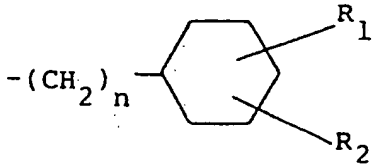
(I)

wherein:

$B^{(+)}$ is the imidazolium or C- or N-substituted imidazolium cation, lysine or similar basic aminoacids or methyl-glucamine;

R represents:

- hydrogen or a linear C_1-C_{25} alkyl chain, optionally substituted by one or more chlorine or fluorine atoms, free, etherified or esterified hydroxy groups, carboxy, carboxyalkyl, aminocarbonyl or N-substituted aminocarbonyl groups, one or more of the $-CH_2-$ groups being optionally substituted by keto groups;
- a chain of formula:



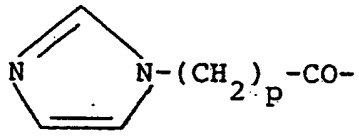
wherein n is an integer from 1 to 10 and R_1 and R_2, which may be the same or different, are H, halogens, $-OR_3$ or $COOR_3$ groups wherein R_3 is hydrogen or C_1-C_5 lower alkyl;

- a chain of formula:

$-(CH_2)_m-Het$

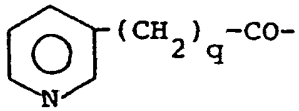
wherein m is an integer from 0 to 20 and Het is an optionally substituted 5- or 6-membered heterocyclic group containing one or more N, O or S atoms such as pyrrole, pyridine, furan, pyran, thiophene, oxazole, isoxazole, imidazole, pyrazole, thiazole groups;

- a chain of formula:



wherein p is an integer from 0 to 16;

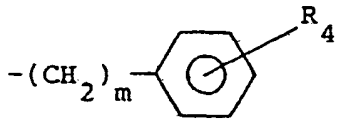
- a chain of formula:



wherein q is an integer from 1 to 16;

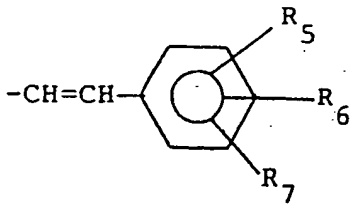
- an aryl or aralkyl residue such as phenyl; phenyl substituted by one or more fluorine or chlorine atoms, fluoroalkyl, alkoxy, alkoxycarbonyl, C_1-C_4 lower alkyl, amino, dialkylamino, hydroxy, cyano groups or by groups of formula $NHCOR_3$ wherein R_3 has the above defined meanings; diphenyl; naphtyl groups;

- a chain of formula:



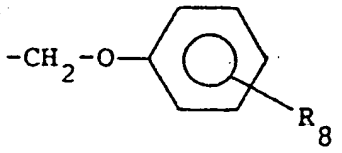
wherein m has the above defined meanings and R_4 is hydrogen or a linear or branched, saturated or unsaturated, C_1-C_{20} alkyl group;

- a chain of the formula:



wherein R_5, R_6 and R_7, which may be the same or different, are H, OR_3 (R_3 having the above defined meanings), NH_2, $NHCOR_3$, chlorine or fluorine atoms, fluoroalkyl groups;

- a chain of formula:



wherein R_8 is hydrogen, lower alkyl, fluorine or fluoroalkyl;

- a linear or branched chain of the formula:

$$-(CH_2)_n-X-\underset{\displaystyle R_3}{\underset{|}{CH}}-(CH_2)_{n'}-Y-$$

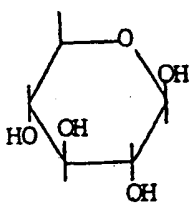
wherein R_3 and n have the above defined meanings, n' is an integer from 1 to 10 and X and Y are an oxygen, nitrogen, sulphur atom or a CH_2 group;

- a chain of formula:

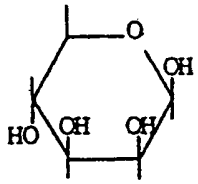
$$-(CH_2)_r-S-R_3$$

wherein r is an integer from 1 to 3 and R_3 has the above defined meanings;

- an aminoacid residue, namely L-leucyl, α or γ-L-glutamyl- in a free form or protected with the conventional amine protecting group, such as BOC;
- an Arg-Pro-D(Phe) chain or the like;
- an uronic residues of formula:



or



2. Compounds according to claim 1 wherein B is imidazolium or 2-aminoimidazolium.

3. A compound according to claim 1 wherein B is the imidazolium residue and the anionic component is selected in the group consisting of:

- 2-hydroxy-5-(2,4-dichlorobenzoyl)amino-benzoic acid;
- 2-hydroxy-5-hexadecanoyl-amino-benzoic acid;
- 2-hydroxy-5-isovaleroyl-amino-benzoic acid;
- 2-hydroxy-5-cyclohexylacetyl-amino-benzoic acid;
- 2-hydroxy-5-succinoyl-amino-benzoic acid;
- 2-hydroxy-5-benzoyl-amino-benzoic acid;
- 2-hydroxy-5-salicyloyl-amino-benzoic acid;
- 2-hydroxy-5-/4-(2',4'-difluorophenyl)salicyloyl-amino/-benzoic acid;
- 2-hydroxy-5-(N-ethoxycarbonyl)glycyl-amino-benzoic acid;
- 2-hydroxy-5-/(6-ethoxycarbonylamino)capropylamino/-benzoic acid;
- 2-hydroxy-5-(N-ethoxycarbonylglutamoylamino)-benzoic acid;
- 2-hydroxy-5-glucuronyl-amino-benzoic acid;
- 2-hydroxy-5-formyl-amino-benzoic acid;
- 2-hydroxy-5-stearoyl-amino-benzoic acid;
- 2-hydroxy-5-(4-methoxy)benzoyl-amino-benzoic acid;
- 2-hydroxy-5-(4-eptyloxy)benzoyl-amino-benzoic acid;
- 2-hydroxy-5-(4-fluoro)benzoyl-amino-benzoic acid;
- 2-hydroxy-5-acetyl-amino-benzoic acid;

- 2-hydroxy-5-linoleyl-amino-benzoic acid;
- 2-hydroxy-5-arachidyl-amino-benzoic acid;
- 2-hydroxy-5-arachidonyl-amino-benzoic acid;
- 2-hydroxy-5-(2,6-difluoro)benzoyl-amino-benzoic acid;
- 2-hydroxy-5-(3,5-difluoro)benzoyl-amino-benzoic acid;
- 2-hydroxy-5-(4-cyclohexyl-butanoyl)-amino-benzoic acid;
- 2-hydroxy-5-(2-(3-pyridyl)-acetyl)-amino-benzoic acid;
- 2-hydroxy-5-(4-phenyl-benzoyl)-amino-benzoic acid;
- 2-hydroxy-5-(m-trifluoromethyl-cinnamoyl)-amino-benzoic acid;
- 2-hydroxy-5-(8-(1-imidazolyl)-octanoyl)-amino-benzoic acid
- L-leucyl-5-amino-salicylic acid;
- γ-L-glutamyl-5-amino-salicylic acid;
- aceto acetyl-5-amino-salicylic acid.

4. As a novel compound, a compound selected in the group consisting of:

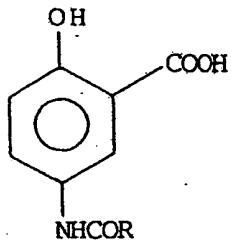
- 2-hydroxy-5-(4-cyclohexyl-butanoyl)-amino-benzoic acid;
- 2-hydroxy-5-(2-(3-pyridyl)-acetyl)-amino-benzoic acid;
- 2-hydroxy-5-(4-phenyl-benzoyl)-amino-benzoic acid;
- 2-hydroxy-5-(m-trifluoromethyl-cinnamoyl)-amino-benzoic acid;
- 2-hydroxy-5-(8-(1-imidazolyl)-octanoyl)-amino-benzoic acid.

5. A process for the preparation of compounds of formula I characterized in that the 2-hydroxy-5-amino-benzoic acid is reacted with acyl chlorides or anhydrides of acids having formula RCOOH, wherein R has the above defined meanings, and that the obtained N,O-diacyl derivatives are hydrolyzed in the presence of imidazole and subsequently

reacted with the base B.

6. Pharmaceutical compositions endowed with antiinflammatory, antiaggregant, antithrombotic activity containing as the active principle one or more of the compounds of claims 1-4.

7. Pharmaceutical compositions endowed with antiinflammatory, antiaggregant, antithrombotic activity containing as the active principle at least a compound of formula:



wherein R has the above defined meanings or of pharmaceutically acceptable salts thereof.

8. A method of treatment of inflammatory, thrombotic or hyperaggregating conditions in a living subject characterized by administering to said living subject a composition of claims 6-7.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 85/00645

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 D 233/56; C 07 C 103/46; C 07 D 213/56; C 07 H 13/12
C 07 D 233/58; C 07 C 103/50; C 07 C 103/82; A 61 K 31/60

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 07 D 233/00; C 07 C 103/00; C 07 D 213/00; C 07 H 13/00; A 61 K 31/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	EP, A, 0124791 (AMERICAN CYANAMID) 14 November 1984 see claims	1,6-8
X,Y	FR, M, 4546 (ROCAL) 2 November 1966 see abstract	1,6-8
Y	Chemical Abstracts, vol. 92, no. 19, 12 May 1980 (Columbus, Ohio, US) see page 578, abstract no. 163724c; & JP, A, 79125632 (Hisamitsu Pharmaceutical Co., Inc.) 29 September 1979	1,6-8
X	DE, A, 2031227 (MERCK) 7 January 1971 see claims and pages 1-4	1,4-8
Y	FR, A, 2214476 (KISSEI YAKUHIN KOGYO KABUSHIKI KAISHA) 19 August 1974 see claims	6-8

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th March 1986	09 APR 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/EP 85/00645 (SA 11505)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/03/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0124791	14/11/84	JP-A-	59231058	25/12/84
		US-A-	4536346	20/08/85
FR-M- 4546		None		
DE-A- 2031227	07/01/71	NL-A-	7008623	29/12/70
		FR-A-	2053015	16/04/71
		GB-A-	1268465	29/03/72
		US-A-	3632760	04/01/72
		CH-A-	536278	30/04/73
		US-A-	3674844	04/07/72
FR-A- 2214476	19/08/74	NL-A-	7400754	22/07/74
		BE-A-	809935	16/05/74
		DE-A,B,C	2402398	08/08/74
		AU-A-	6461374	17/07/75
		US-A-	3940422	24/02/76
		GB-A-	1446141	18/08/76
		AT-B-	333726	10/12/76
		US-A-	4070484	24/01/78
		CH-A-	613442	28/09/79
		JP-A-	49093335	05/09/74
		CH-A-	615152	15/01/80
		SE-B-	411117	03/12/79